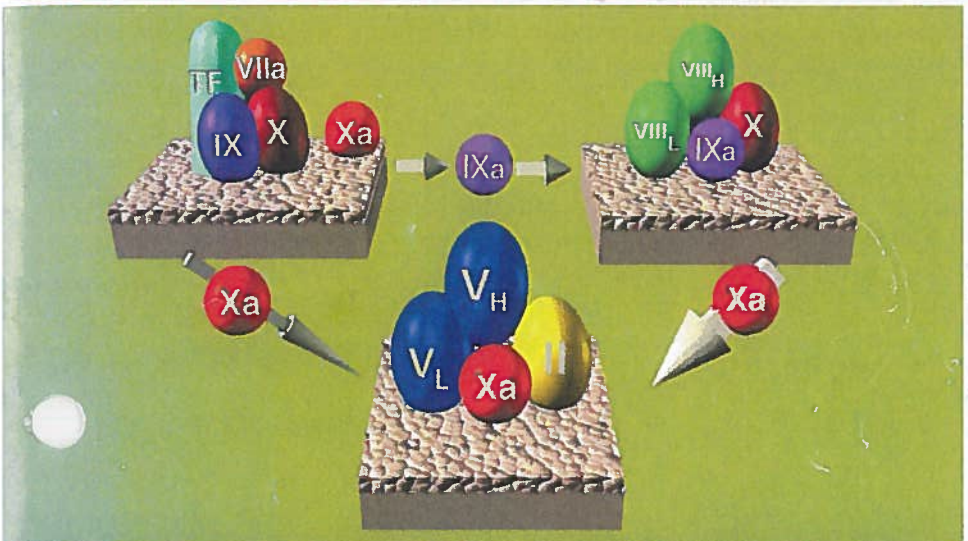
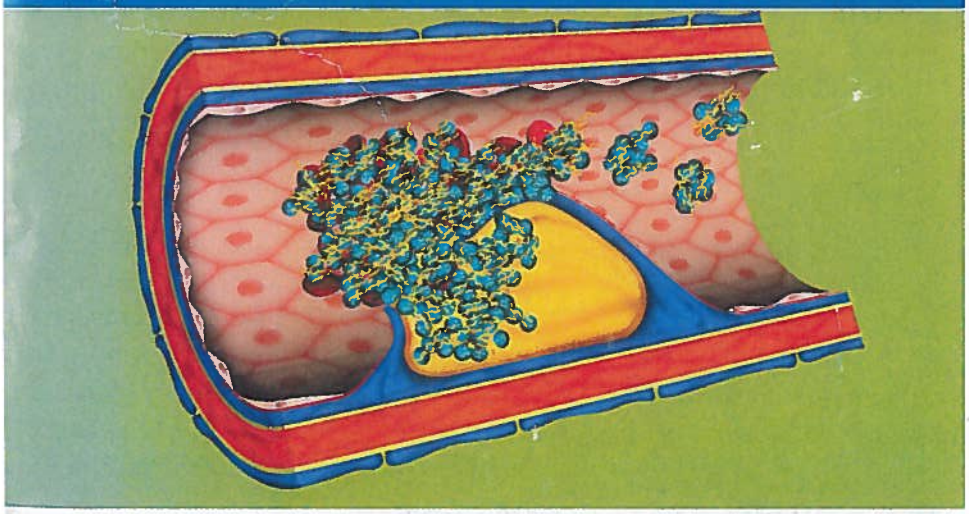
THROMBOSIS PRIMER

PATHOPHYSIOLOGY OF CLOTTING AND ACUTE CORONARY SYNDROMES



A Continuing Medical Education Monograph for Practicing Physicians



DEVELOPED BY THE INTERNATIONAL THROMBOSIS EDUCATION INITIATIVE™

IN COLLABORATION WITH THE AMERICAN COLLEGE OF CARDIOLOGY, THE AMERICAN SOCIETY OF HEMATOLOGY AND THE NATIONAL HEART, LUNG AND BLOOD INSTITUTE

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PREFACE

"In this desperate situation, I wondered if a disturbance of the nourishment of the heart could explain such a condition, and whether this could have been caused by a thrombotic occlusion of at least one coronary artery."

Adam Hammer, 1818-1878

Ever since this critical observation was made in 1877, one year before Hammer's death, the role of thrombosis as a cause of acute myocardial infarction has been the object of numerous studies...and debates, if not controversies. While today thrombosis is widely accepted as the precipitating event of acute coronary syndromes, the road to this consensus was long and sometimes tortuous. Just consider what was said in 1925:

"...it is most reasonable to regard a thrombus not as an entity, but merely as one of the end results of coronary disease..." (1)

and again in 1974:

"In conclusion, there is substantial evidence that acute thrombosis formation does not precipitate fatal ischemic heart disease." (2)

The views on this matter began to change rapidly thereafter following the publication of reports which established that thrombolytic (or fibrinolytic) agents reduced mortality in acute myocardial infarction patients. One of the first such observations was that of Chazov et al who demonstrated coronary recanalization using streptokinase (3). This was followed a few years later by the report of the European Cooperative Study Group for Streptokinase Treatment in Acute Myocardial Infarction (4).

In the ensuing years, the concept of thrombosis as the hallmark of acute coronary syndromes has developed and eventually fully materialized. Undoubtedly, this realization came from the convergence of two forces: first and most important, the understanding of the thrombotic process and second, the consequent development of thrombolytic agents whose efficacy has now been widely demonstrated.

Today, one cannot overstate the importance, not to say the essentialness, of thrombolytic therapy in acute coronary events. Thus, it is no surprise that the scientific community and the pharmaceutical industry continue to search for new and better agents. One may then ask: "Is thrombolytic therapy the only strategy?" The answer is, of course, negative and, indeed, the power of interventional approaches has been extensively demonstrated by experience as well as by randomized studies.

No doubt, cardiologists have available an armamentarium of approaches from which to choose, an important benefit to patients. This is amply shown by the decline of the coronary disease death rate that we have witnessed during the last decade. However, the full potential of therapies can be realized only if their mechanism of action is well understood.

This publication, "Thrombosis Primer: Pathophysiology of Clotting and Acute Coronary Syndromes," gives the reader the opportunity to uncover the pathology of coronary events and mechanisms of action of the therapies that are available. It results from a joint effort of two eminent professional organizations, the American College of Cardiology and the American Society of Hematology. The contributors who were chosen to develop this educational monograph are experts whose scientific and clinical achievements are recognized worldwide.

The National Heart, Lung and Blood Institute has the mandate to disseminate to the practicing community and to the public the outcomes of the research it supports. Thus, it welcomes the opportunity to be a cosponsor of this document which furthers its mission.

– CLAUDE LENFANT, M.D.
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PLATELETS IN COAGULATION

Introduction

Thrombosis is the precipitating factor in nearly all cases of acute coronary occlusion and thus is the most immediate problem leading to death from coronary artery disease in the United States. The importance of understanding the process of thrombosis and ways to intervene to prevent thrombosis or to dissolve thrombi cannot be overemphasized.

Homeostasis and thrombosis are the end results of a carefully orchestrated series of enzymatic reactions, which are often grouped into discrete units. Separation into these discrete units facilitates understanding of both the molecular mechanisms leading to hemostasis and thrombosis, and it is useful to identify the potential sites of therapeutic intervention in the coagulation pathways. The following discussion will also be grouped into such units for convenience. However, the processes of hemostasis and thrombosis *in vivo* cannot be separated into discrete units, as each of these units is ongoing at any given time. It is only on a quantitative basis that the units can be grouped separately. Thus, platelet activation, the generation of thrombin, and fibrinolysis are all ongoing at any given moment. It is the relative rates and thus the balance of individual reactions at any given time, reflecting also the balance of various activators and inhibitors, that facilitates or retards these reactions, which, in turn, dictate the rate at which a blood clot or a thrombus forms or their dissolution occurs.

Thrombosis itself can be considered as "pathological hemostasis." There is a close relationship between the normal and desirable homeostatic process of blood coagulation and hemostasis and the pathologic event of thrombosis, which cuts off the flow of blood to vital organs and can result in death and/or severe organ dysfunction and disability. The relationship between normal hemostasis and pathologic thrombus formation is so intimate that thrombosis can also be defined as hemostasis occurring at the wrong time or in the wrong place. Thrombosis is generally regarded as an acute and often catastrophic event. However, in the case of atherosclerosis, the pathologic process that leads to the acute thrombotic event and coronary occlusion has a background of ongoing thrombosis for many years and thus thrombosis need be viewed as a continuum. It is therefore important to point out that the mere presence of a non-occlusive atherosclerotic plaque or even plaque rupture and the continued exposure of a thrombogenic surface to flowing blood may not necessarily restrict blood flow to vital organs. The continuous nature of thromboses is often not recognized. It has become universally recognized that the critical event is the formation of an occlusive thrombus, but this view overlooks the contribution of ongoing thromboses prior to the devastating event of acute coronary thrombosis and coronary occlusion.

Recent clinical trials in which antiplatelet agents were used to prevent arterial thrombus formation clearly demonstrate the importance of blood platelets in thrombogenesis and confirm the view that antiplatelet drugs have the potential to maintain the patency of diseased arteries. However, it is equally clear that the resolution of arterial thrombus involves the dissolution of fibrin, and thus clearly indicates the importance of thrombosis, fibrin formation, and fibrinolysis in maintaining vessel patency. Again, although blood coagulation is normally considered as a series of distinct steps, it is the sum of the ongoing processes that determines the outcome of coagulation and whether or not a pathological thrombus will form.

Although the clinical studies are new, the recognition of the multifaceted pathogenesis of thrombosis is long standing. Virchow, in his classic studies of vascular pathology carried out over a century ago, recognized that thrombosis requires changes in three constituents: the vessel wall, blood flow, and blood coagulability. It is now recognized that circulating blood platelets provide the first line of defense when the integrity of the endothelial cell lining of blood vessels is perturbed. Within seconds of injury, platelets accumulate at the site of injury. These reactions result in the development of a multicellular platelet plug, the first defense against vessel wall hemorrhage and frequently a first step for thrombosis. The platelet plug provides a mechanical barrier preventing damaged wall hemorrhage as well as a nidus for the subsequent deposition of fibrin strands generated by the parallel activities of the plasma coagulation system leading to the formation of the clot itself.

Formation of the "Normal" Hemostatic Plug Adhesion

Platelets circulate within vessels as disc-shaped cells and do not interact with normal endothelial cells. Platelets rapidly adhere to damaged endothelium or other components of the vessel wall. In the platelet-endothelium interaction, two processes are of fundamental importance: the platelet-collagen interaction and the interaction of platelets with von Willebrand factor. When exposed to damaged endothelial barrier, platelets rapidly adhere to the collagen and other constituents in the subendothelium. Platelets adhere to collagen through specialized receptors (Figure1). The collagen receptor on platelets (technically known as the $\alpha_2\beta_3$ integrin) is actually composed of two chains, α_2 and β_3 , which span the platelet membrane. The binding of platelets to the vessel wall via the collagen receptor is weak and adherent platelets are readily detached by the shear forces generated

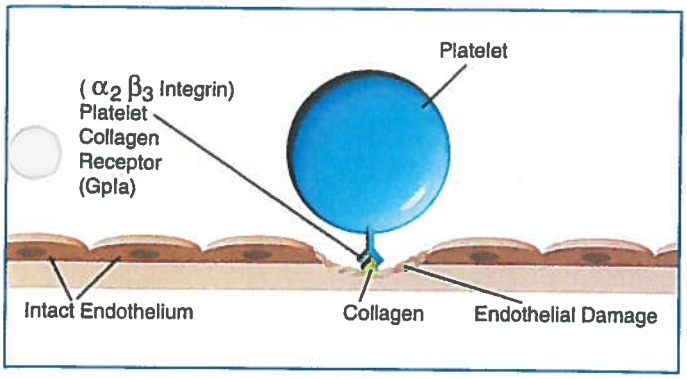

Figure 1. Platelet Adhesion to Collagen

in rapidly moving arterial blood. The critical second interaction of the platelet with the vessel wall involves a complex plasma protein called the von Willebrand factor (vWF) (Figure 2). vWF interacts with a second receptor that is unique to the platelet and is known as platelet glycoprotein Ib, IX, and V complex. This receptor also

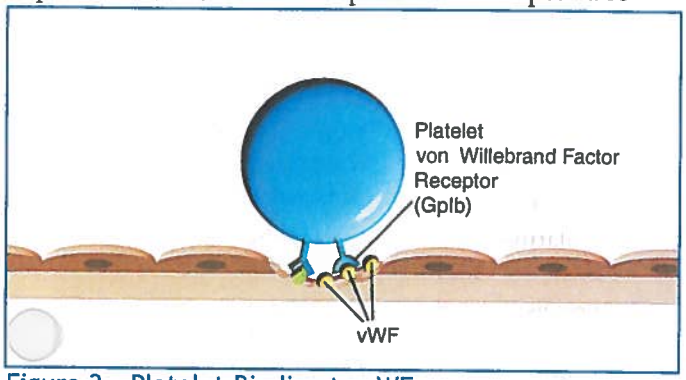

Figure 2. Platelet Binding to vWF

spans the platelet membrane and, as the name implies, is composed of three (non-integrin) proteins: platelet glycoproteins Ib, IX and V (the GpIb/IX/V complex). vWF interacts with a discrete protein sequence (a domain) in the GpIb chain through a discrete domain in vWF itself, known as the A1 domain. vWF also binds to collagen through a portion of the vWF protein known as the A3 domain. The structures of these interactive domains in these proteins are quite similar and are members of a large family of protein domains known as "A domain motifs", which are present in many adhesive proteins and their receptors. This motif clearly plays a critical role in the adhesive events of platelets to the vessel wall, since vWF-mediated adhesion permits platelets to remain attached despite the high shear forces in flowing blood.

The concept of these domains is important, as these are sites of the functional interactions of these proteins, and therefore these domains serve as targets for antithrombotic agents to disrupt the functional interactions of these proteins.

Activation and Secretion

As part of their adhesion to surfaces, platelets are activated, undergo changes in their shape, and release of bioactive proteins and small molecules. These constituents play important roles in hemostasis, inflammation, and vascular remodeling. The activation process is accompanied by the expression of additional receptors that lead to the generation of thrombin and fibrinogen-mediated platelet aggregation. In addition, the shear stress of circulating blood alone can activate adherent platelets.

Activation of platelets through these receptors signals pathways in the platelet that cause shape change and secretion. One of the most important of these pathways leads to generation of the reactive arachidonic acid metabolite thromboxane A_2, a potent vasoconstrictor and platelet agonist. Thromboxane A_2, therefore, can contribute to thrombosis, it also amplifies the effect of platelet adhesion on aggregation. Multiple other stimuli can activate platelets by interacting with other platelet receptors, including $\alpha_2\beta_3$ integrins and the Ib, IX, and V complex. The platelet also has receptors for thrombin, the adenine nucleotide ADP, as well as α_2-adrenergic, vasopressin, and serotonin (Figure 3). Since the activated platelet is one of the essential events leading to thrombosis, these additional receptors and the events they trigger also become targets for therapeutic intervention.

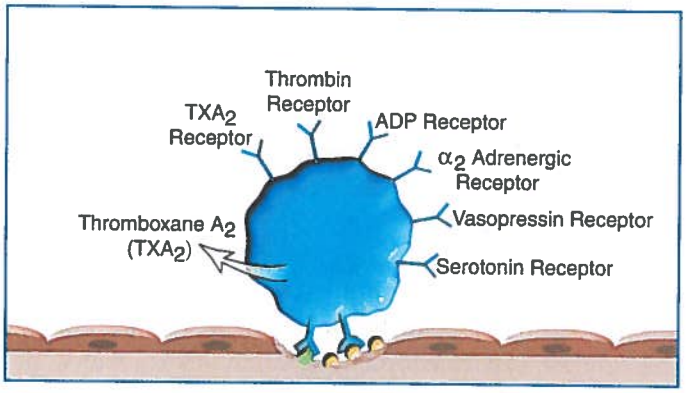

Figure 3. Platelet Receptors

As noted earlier, most thrombotic events are not clinically detectable and are ongoing events as atherosclerosis progresses. In addition to the small molecules released by platelets when they are activated, there are a number of proteins that also interact with the vessel wall. These proteins include various growth factors and chemoattractants with differing specificities towards endothelial cells, smooth muscle cells, fibroblasts, and macrophages. These molecules are very important since their targets are cells of the blood vessel wall, such as the endothelium and the smooth muscle cells, whose attraction to sites within both the tunica media and tunica intima are major contributors to the proliferative phase of atherosclerosis.

Aggregation

Platelet aggregation is another vital step in the events leading to arterial thrombosis. This process is mediated by the platelet protein GpIIb/IIIa (integrin $\alpha II_b\beta_3$),

which is altered during platelet aggregation, enabling it to bind fibrinogen (Figure 4). Fibrinogen is an abundant plasma protein that interacts through the amino acid sequence RGD (for arginine, glycine, and aspartate).

The importance of platelets is underscored by the severe bleeding diathesis present in syndromes in which platelet aggregation is limited such as Glanzmann's thrombasthenia, von Willebrands' disease, and Bernard Soulier Syndrome.

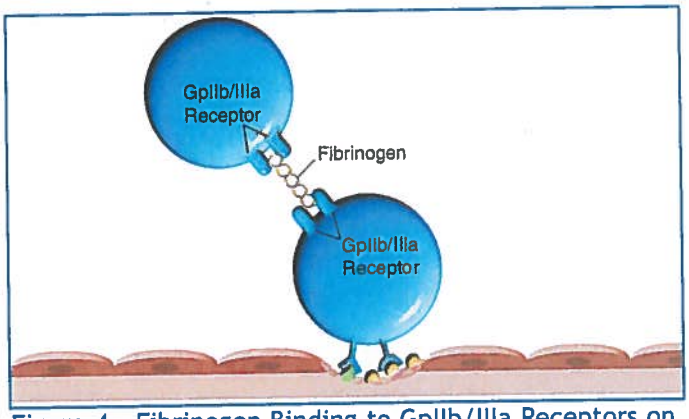

Figure 4. Fibrinogen Binding to GpIIb/IIIa Receptors on Platelets

Platelets and Arterial Thromboembolism

Although platelet thrombi and hemostatic plugs arise via the same general mechanisms, there are some unique features operative in thrombosis, which relate to the state of the vessel wall and the presence of unique thrombogenic stimuli. For example, changes in the composition and/or size of atherosclerotic plaques make them "unstable", prone to rupture, and therefore thrombogenic. Plaques are also rich in Tissue Factor (discussed below), which may enhance local generation of thrombin and which is also a potent activator of platelets. Partial obstruction of the vessel and shear stress leading to turbulent blood flow enhances platelet activation. In addition, the protective effects of adjacent endothelium may be absent due to the lack of normally functioning endothelium. Artificial surfaces, such as those in vascular prostheses (stents, Dacron grafts, etc.), also present highly thrombogenic surfaces with exposure for their entire intravascular life span.

The stability of a thrombus is in part due to its content of fibrin. Platelet thrombi formed with little fibrin are inherently unstable and portions may detach and embolize to distal parts of the circulation. The evolving nature of thrombi both enhances their ability to cause events, frequently microcirculatory, and makes them more amenable to therapeutic interventions. For example, it may only be necessary to slow down or incompletely inhibit the rate of platelet accretion into an adherent platelet monolayer to have a significant therapeutic effect.

The Use of Antiplatelet Drugs to Treat and Prevent Thrombosis

We are in the midst of an antithrombotic/anticoagulant revolution. The two most widely utilized anticoagulant agents, heparin and the coumarins, were introduced into clinical practice over half a century ago. Although both agents are effective in reducing the rate and extent of prothrombotic reactions, neither is particularly effective against the platelet-based arterial thrombosis. Remarkably, the first effective antiplatelet agent introduced into clinical practice is the common and widely used anti-inflammatory agent and anti-pyretic, acetylsalicylic acid—aspirin. Aspirin inhibits platelet activation by irreversibly acetylating the enzyme cyclooxygenase, a key enzyme in the production of thromboxane A_2 from arachidonic acid. Although aspirin is a weak agonist by *in vitro* tests, clinical trials have shown it has a dramatic clinical effect. The widespread success of aspirin has led to the development of additional antiplatelet agents. The most interesting now are a group of agents directed against the platelet GpIIb/IIIa complex. These agents, by interfering with fibrinogen binding, virtually eliminate platelet thrombus formation, leaving behind only an innocuous adherent platelet monolayer.

Other targets for intervention are the platelet receptors responsible for platelet activation (e.g., the ADP or thrombin receptors). Clinically effective agents include ticlopidine and clopidogrel, which are thought to exert their effects by blocking the platelet ADP receptor. They are effective agents, can be taken orally, and work in certain settings in which aspirin has failed. Newer agents, which offer great promise, are now being tested. The explosion of new agents and new targets available now or in the near future for clinical uses underscores the importance of understanding the basis of clot and thrombus formation.

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THE PHASES OF COAGULATION

The generation of thrombin following vascular injury occurs in two waves of very different magnitude. A tiny amount of thrombin is produced during the *Initiation Phase* of the reaction. During this phase, picomolar (10^{-12}M) concentrations of thrombin are produced that contribute to the generation and assembly of the catalysts that provide the subsequent explosive thrombin generation that erupts during the *Propagation Phase* of blood coagulation. During this latter phase of the reaction, thrombin is produced at micromolar (10^{-6}M) concentrations, a million-fold higher concentration than those produced during the *Initiation Phase.*

In blood, clotting occurs at the inception of the *Propagation Phase.* Since the endpoint for many laboratory tests of coagulation is the clot, such tests only measure alterations in the *Initiation Phase.*

These two phases of coagulation are regulated differentially by the inhibitory systems present in blood plasma, blood cells, and vascular tissue. High-affinity inhibitors, which are at low abundance, are more important in blocking the *Initiation Phase.* Other inhibitors that have lower affinity but are present at higher abundance are more important in down-regulating the *Propagation Phase* of the reaction.

Threshold of Coagulation

The blood inhibitory systems consist of components of higher anticoagulant potential than those expressed by the procoagulants normally present in blood. Thus, a stimulus must reach a sufficient level to ignite the clotting reaction. The interplay between pro- and anticoagulant systems in blood determines thresholds for the coagulation response to be initiated. This interaction is vital in maintaining the balance between blood fluidity and protection from bleeding.

The inhibitory elements that attenuate the propagation phase of clotting counter the activities of the blood plasma proteins responsible for the generation of thrombin. This occurs during the *Attenuation Phase,* which blocks progression the blood coagulation process with respect to thrombin generation and clot formation.

The barrier to blood flow, the blood clot, is a complex array in which the principal constituents are the activated aggregated blood platelets and fibrin, the cross-linked product of thrombin cleavage of fibrinogen. These reaction products, which provide the temporary physical barrier to blood loss in case of hemorrhage or block blood flow in thrombosis, are removed by the actions of the fibrinolytic pathway during the *Elimination Phase* of coagulation in which the enzyme plasmin dissolves the clot. The damaged vascular elements are replaced by newly synthesized connective tissue and cellular components during the *Repair Phase.* Although the blood coagulation and vascular-repair phases are presented sequentially, these processes overlap and are integrated.

Cells Involved in Coagulation

A number of cell types contribute to the coagulation process. Monocytes, platelets, vascular endothelial cells, and the surrounding vascular muscle cells are the major contributors, with each performing specialized functions. Under ordinary circumstances, a low level coagulation but without significant clot formation occurs in the circulation as evidenced by the detection of circulating protein fragments associated with the coagulation/fibrinolysis processes. However, if the endothelial integrity barrier is broken, the reactions leading to substantial, local thrombin generation are initiated. Intravascular damage can occur due to the rupture of an atherosclerotic plaque or a penetrating injury. In either case, platelets adhere and aggregate to surround and cover the site of vascular damage, with the resulting mass of perivascular cell tissue, damaged endothelial cells, and adherent platelet membranes providing a platform for coagulation and the generation of thrombin and fibrin.

Many molecules influence the coagulation of blood. In this review, the focus is on those most relevant to hemorrhagic and thrombotic disease.

Initiation

Thrombin generation begins with the exposure of tissue factor to blood. Tissue factor is an integral membrane protein that is normally expressed on extravascular tissue and can be induced to be expressed on monocytes and endothelial cells. The tissue factor exposure to plasma leads to a chain reaction that ultimately generates thrombin. The key processes in this reaction are activation of plasma-derived proenzymes and procofactors that form the membrane-bound enzymes, leading to the generation of thrombin.

The proenzymes are all vitamin K-dependent proteins that are synthesized in the liver. Following proteolytic activation, the enzymes are short-lived in the circulation; the exception to this rule is factor VIIa. The cleaved form of factor VII (factor VIIa) is an inactive protease that requires binding to tissue factor to reveal its active site. As a consequence, factor VIIa escapes the plasma inhibitors that recognize the active site function, and a small fraction (1-2%) of the total factor VII in blood circulates as factor VIIa. Tissue factor exposed by vascular injury will bind plasma factor VIIa, forming the initial catalyst that

starts the coagulation reaction. The resulting procoagulation system can be represented as a series of three similar vitamin K-dependent enzyme complexes, each composed of a serine protease and a cofactor protein assembled as a complex on a membrane surface. Each complex acts on a membrane-bound substrate (Figure 1).

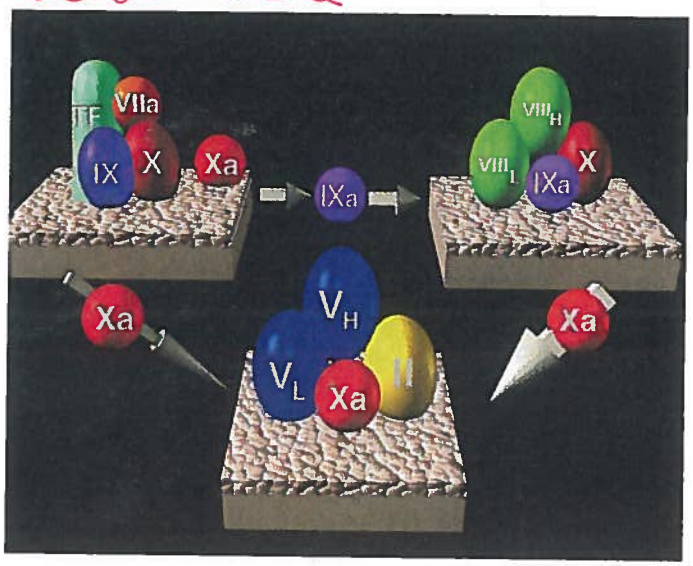

Figure 1.
Each complex is composed of a serine protease (factor VIIa, factor IXa OXa, and factor Xa) bound to a cofactor protein (tissue factor, factor VIIIa and factor Va) bound on a membrane surface. Each complex catalyzes the activation of a vitamin K-dependent zymogen(s) (factor IX, factor X, prothrombin) (II). From the "Coagulation Explosion", Vermont Business Graphics, Kenneth G. Mann, 1997, by permission.

During the Initiation Phase, the principal player is the "extrinsic factor Xase," a complex composed of cell membrane, tissue factor exposed by vascular damage or cytokine stimulation, and plasma factor VIIa. The plasma factor VIIa-tissue factor complex can activate the proenzymes factor X and factor IX to their respective products, factor Xa and factor IXa. Initially, the activation of factor X is greater than the activation of factor IX; thus, the initial product is predominantly factor Xa. Once generated, however, factor Xa feeds back to increase factor IXa formation. Factor Xa bound to the membrane will convert factor IX to a form that can bind to tissue factor-factor VIIa as a substrate and be converted to factor IXa. Factor Xa bound to the membrane can also activate more factor VII to the active two-chain factor VIIa.

The tissue factor pathway inhibitor (TFPI) is present at low abundance in blood but has high affinity for the tissue factor-factor VIIa-factor Xa product complex. Thus, as the coagulation reaction proceeds, the tissue factor-factor VIIa complex generates factor Xa and factor IXa, with the reaction terminated by the formation of the factor Xa-factor VIIa-tissue factor-TFPI complex (Figure 2).

Xa facilitate activation of IX by TF:VIIa

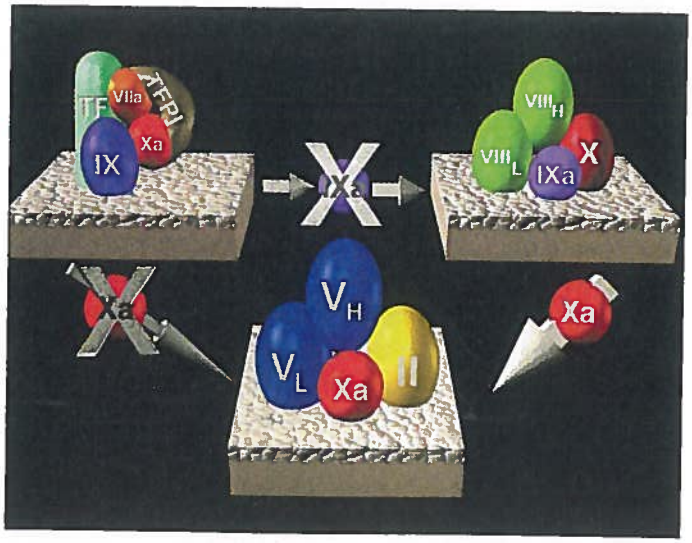

Figure 2.
The factor VIIa tissue-factor complex is inactivated by the binding of TFPI to the factor VIIa-tissue-factor Xa product complex, eliminating the generation of factor IXa and factor Xa by this catalyst. The resulting effect is that factor Xa generation is catalyzed by the factor VIIIa-factor IXa complex. From the "Coagulation Explosion", Vermont Business Graphics, Kenneth G. Mann, 1997, by permission.

The initiation reaction leads to the generation of small amounts of factor Xa and factor IXa. The former participates in the formation of "prothrombinase," the factor Xa-factor Va-membrane complex that activates prothrombin to thrombin. Plasma factor V reversibly binds to membrane sites provided by the activated platelet or by vascular damage and is activated to factor Va by factor Xa on a membrane surface and by thrombin. Factor Xa binds to factor Va and activates membrane-bound prothrombin to thrombin. Once thrombin is formed, it can rapidly activate more factor V to factor Va, leading to a burst of factor Va during the Initiation Phase of the coagulation reaction. This initial amount of thrombin also catalyzes the activation of factor VIII (antihemophilic factor) to factor VIIIa that permits formation of what is referred to as "intrinsic factor Xase", which is composed of factor VIIIa and factor IXa bound to an active membrane. This catalyst efficiently activates factor X to factor Xa. The resulting burst of factor Xa, which occurs from catalysis by the intrinsic factor Xase, results in the Propagation Phase of the reaction.

Propagation

During the Propagation Phase of coagulation, the extrinsic factor Xase is neutralized by TFPI (Figure 2), and the thrombin generation reaction is continued by the intrinsic factor Xase and prothrombinase. The factor IXa-factor VIIIa complex activates factor X to factor Xa much more efficiently than the factor VIIa-tissue factor complex; therefore, the bulk of the factor Xa generated comes from the intrinsic factor Xase. These two complexes generate bursts of factor Xa and thrombin that lead to a "crescendo-like" effect on clotting as more and more complexes are assembled and generate thrombin.

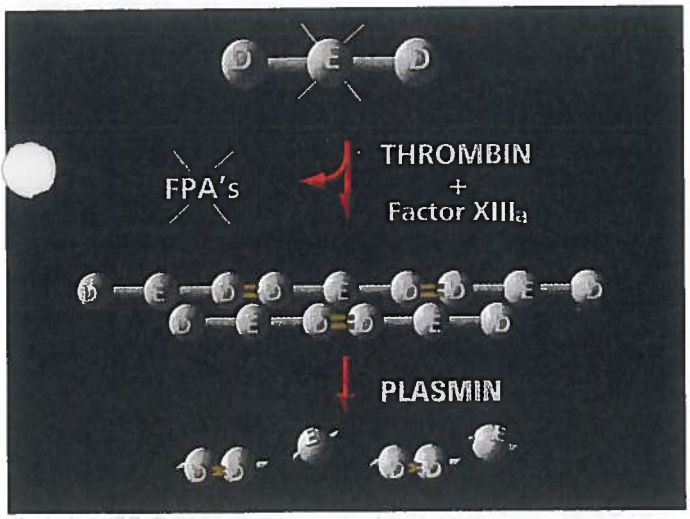

Figure 3.
The fibrinogen molecule is composed of three major domains, D, D, and E and converted to insoluble fibrin by thrombin cleavage of fibrinopeptides (FPA's). Thrombin also activates factor XIII to factor XIIIa. This enzyme produces cross-links between the adjacent D domains in the insoluble fibrin clot. Plasmin generated from plasma plasminogen by either urokinase or tissue plasminogen activator cleaves between D and E domains to result in the fibrin degradation products (FDP's). From the "Coagulation Explosion", Vermont Business Graphics, Kenneth G. Mann, 1997, by permission.

Once generated, thrombin activates additional platelets and, by cleaving fibrinopeptides, transforms fibrinogen to fibrin, which forms clumps with activated platelets already at the site of injury. Fibrinogen is the plasma precursor of fibrin, the principal plasma constituent of the clot (Figure 3). However, fibrinogen plays many roles in the coagulation and fibrinolytic/repair processes. It is an important component of the platelet aggregation process and a major contributor to the activation of the fibrinolytic system. The molecule is composed of six chains arranged as three symmetrical pairs of chains. Short NH_2-terminal peptide regions of the molecules Aα and Bβ chains (the fibrinopeptides) are released by thrombin, and the resulting fibrin molecules form an insoluble gel through aggregation. This noncovalently bound fibrin gel is further stabilized by the introduction of covalent cross- links that render the fibrin clot insoluble. The cross-links are between selected glutamyl and lysyl residues present in the fibrinogen molecule. Factor XIIIa, which catalyzes the crosslinking process, circulates in plasma in a precursor form (factor XIII), which is cleaved to the active species by thrombin.

It should be recognized that the ultimate magnitude of the reaction depends on the extent of activated membrane exposed in the damaged tissue and the aggregated platelets, which are accumulated in the region of the wound.

Termination

Termination of coagulation is accomplished by a collection of stoichiometric and enzymatic processes that inactivate the proteins of the procoagulant complexes and inhibit the residual enzymes. This occurs through the stoichiometric inhibitors antithrombin III (AT-III) and TFPI and through the protein C system. Thrombin released from prothrombinase binds to endothelial cell-bound thrombomodulin (Tm) and converts the vitamin K-dependent proenzyme protein C to activated protein C (APC) on the membrane surface (Figure 4). Once thrombin is bound to thrombomodulin, its proteolytic specificity is altered to recognize the zymogen protein C, and, once bound, thrombin will no longer recognize fibrinogen, platelets, or factor V. The activated protein C targets the membrane-bound factor VIIIa and factor Va molecules. The concentration of this natural anticoagulant enzyme determines the lifespan of these essential elements of the procoagulant complexes. Since the procoagulant complexes (Figure 1) converting factor X and prothrombin to their respective products are reversibly bound, activated protein C can interchange with factor Xa and factor IXa at their respective binding sites with the factor Va and factor VIIIa molecules (Figure 4). Subsequent cleavages in the heavy chain regions of both of these molecules leads to their inactivation so that they can no longer bind their respective enzymes or substrates. Antithrombin III forms complexes with the remaining factor IXa, thrombin, and factor Xa, neutralizing all the procoagulant enzymes in the system (Figure 4).

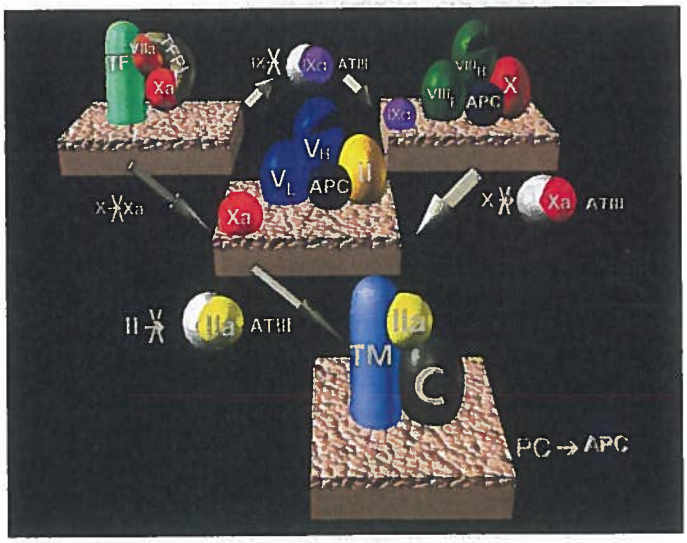

Figure 4.
Thrombin binds to vascular thrombomodulin (TM) and activates the zymogen protein C to activated protein C (APC) which down-regulates coagulation by binding competitively with factor Xa and factor IXa and cleaving factor Va and factor VIIIa inactivating these molecules. Antithrombin III inhibits thrombin, factor Xa and factor IXa. From the "Coagulation Explosion", Vermont Business Graphics, Kenneth G. Mann, 1997, by permission.

Elimination

The coagulation plug is a complex composite, with the principal structural components being aggregated platelets and cross-linked fibrin. Other constituents are entrapped within this matrix, including other plasma proteins and blood cells. This temporary seal is eliminated as the reconstruction of a stable vascular structure

occurs. The principal enzyme acting to destroy the platelet-fibrin plug, plasmin, is generated from its plasma precursor, plasminogen, by the action of two enzymes, urokinase (UK) and tissue plasminogen activator (t-PA), both of which are secreted by the vascular endothelial cell. The secretion of these two enzymes is regulated by cellular cytokines and components produced during clotting, including thrombin. The inhibitor of the fibrinolytic enzymes, plasminogen activator inhibitor I (PAI-1), is also secreted by vascular endothelial cells. The antagonism between plasminogen activator and PAI-1 determines a threshold response to proceed to fibrinolysis in much the same way as the pro and anticoagulants determine thesholds for the clotting process. With sufficient stimulus the tissue plasminogen activators convert soluble plasma plasminogen to the enzyme plasmin, which binds to the fibrin clot and cleaves selected bonds in fibrin, leading to clot dissolution or "fibrinolysis." The process of plasmin digestion of fibrin clots is further regulated by the plasma inhibitor termed α_2-antiplasmin, a process also catalyzed by fibrin.

The terminal products of fibrin clot digestion are the so-called D-dimers, which are produced by plasmin digestion of the cross-linked terminal D domains from adjacent fibrin molecules (Figure 3). These markers are clinically useful in assessing the extent of ongoing fibrinolysis in a thrombotic event and during therapy with infused thrombolytic agents.

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THE ROLE OF LABORATORY TESTING IN UNDERSTANDING, PREDICTING, AND MANAGING THROMBOSIS

INTRODUCTION

Laboratory testing in the areas of coagulation, fibrinolysis, and thrombosis has been characterized over the years by a large array of different tests, many of uncertain application, mostly focused on the diagnosis and management of venous thrombosis. Recent research findings sparked a new area of interest: the prediction of arterial thrombosis; most importantly, myocardial infarction. As a striking example, it has recently been shown that young women who have a relatively common genetic mutation of a key coagulation factor, and who smoke, have a 32-fold increased risk of myocardial infarction over similar women who are without the mutation and do not smoke. Such a large increase in risk, confined primarily to smokers, illustrates two important points: 1) new coagulation and fibrinolysis tests can improve our ability to predict arterial thrombosis, and 2) abnormal concentrations, or forms, of coagulation or fibrinolysis factors may increase risk in the presence of other risk factors, such as smoking.

Despite numerous coagulation and fibrinolysis factors, they can be viewed in a simplified scheme for discussion purposes, such as the "four-quadrant" model proposed in (Figure 1). The general concept is that procoagulant factors are balanced by anticoagulant factors, which drive the formation of a clot. Clot resolution is driven, in turn, by the fibrinolytic factors balanced by antifibrinolytic factors. The fibrinolytic process results in circulating Fibrin Degradation Products (FDPs), which are ultimately cleared from the circulation. Thus, under "normal" conditions, proper balances are necessary to keep the blood clot-free. If the balance is tipped in favor of one quadrant or another, there is a tendency toward either excessive clot formation (e.g., antithrombin deficiency) or excessive fibrinolysis (e.g., pharmacological thrombolytic therapy).

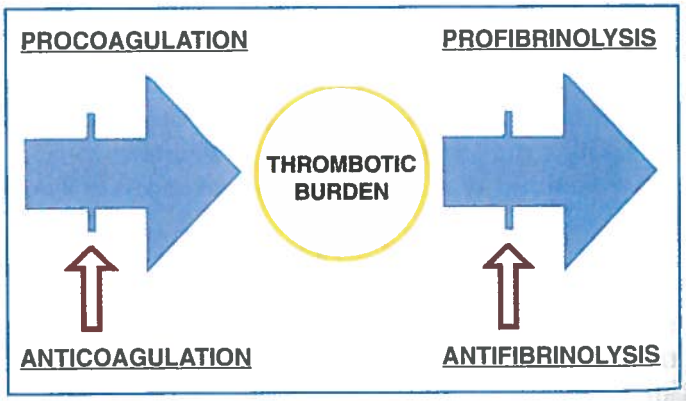

Figure 1.

any clotting factors and processes can be measured in earch settings, but clinically only a few measurements : in common use. These measurements proven clinilly ful in the assessment of clinical thrombotic dise include:

Screening tests, such as the Prothrombin Time (PT), the Activated Partial Thromboplastin Time (aPTT) and the Bleeding Time
Specific factor assays, such as the Fibrinogen assay, the Antithrombin assay, the Protein C assay and the Protein S assay
Assays for products of fibrinolysis, such as the general FDP assay and the specific assay for the FDP known as D-dimer
Assays for heparin monitoring, such as the aPTT and the Activated Clot Time (ACT)

pecial assays are available-factor VIIa (a measure of fac-or VII activation state), the prothrombin activation pep-de fragment F1+2 (a marker of thrombin generation), nd PAI-1 antigen and activity. These special tests help o diagnose particularly complex clinical situations. Recently, several genetic tests have been added to the routine list, such as the test for Factor V Leiden, a common nutation that affects the clearance of the procoagulant actor Factor V, and Prothrombin G20210A, a common nu on associated with higher plasma level of the pro-oagulant factor Prothrombin.

Laboratory Testing and Venous Thrombosis

In virtually all laboratories, standard algorithms are available for assessing venous blood clotting problems. For example, a routine panel of tests ordered on a person presenting an unexplained deep vein thrombosis (DVT) might include some or all of the following tests:

- Complete Blood Count
- PT, aPTT and the Thrombin Time
- Antithrombin, Protein C and Protein S levels
- APC-resistance test and the Factor V Leiden genotype
- Dilute Russell Viper Venom Time and Anticardiolipin Antibodies
- Prothrombin G20210A genotype
- Homocysteine levels

These tests reflect the current state-of-the-art with respect to diagnosis and risk prediction for venous thrombosis. The CBC with blood smear is used to identify myeloproliferative diseases that may be associated with thrombosis. The PT and aPTT are the standard screening tests for abnormalities in the Intrinsic and Extrinsic coagulation pathways. They are particularly sensitive to deficiencies of procoagulant clotting factors; however, such deficiencies are not usually involved in clotting disorders. The Thrombin Time is a test for abnormal Fibrinogen (dysfibrinogenemia), a rare cause of venous clotting.

The majority of venous clotting disorders are caused by deficiencies of natural anticoagulants, by abnormally slow elimination of procoagulants, or by acquired alterations in membrane structure and function. The anticoagulants usually tested are Antithrombin, Protein C and Protein S. Tests for the activities of these factors should be done first, with tests for the antigens done later if activity levels are low. Factor V Leiden is a relatively common (3-7% of most populations) mutation in the procoagulant Factor V that slows down its elimination by the active form of Protein C (APC). The presence of Factor V Leiden is a risk factor primarily for venous thrombosis (as is Protein C deficiency—since the end result is the same, namely, slow clearance of Factor Va), and under some circumstances, arterial thrombosis. Two tests are commonly used to assess Factor V Leiden: the APC-Resistance Test and the Factor V Leiden genotype assay.

Disorders of membrane structure and function that lead to clotting abnormalities occur mainly in the presence of autoantibodies directed against the phospholipids that constitute the membrane assembly sites of the coagulation factors. Antiphospholipid antibodies were first identified in patients with systemic lupus erythematosus, and one class of these autoantibodies has become known as "Lupus Anticoagulants," based on their apparent anticoagulant effect on *in vitro* clotting tests. However, these antibodies are often found in patients with venous thrombosis and, less commonly, arterial thrombosis. A slightly different class of thrombotic autoantibodies includes the anticardiolipin antibodies. Screening for Lupus Anticoagulants is done with the Dilute Russell Viper Venom Time test, while anticardiolipin antibodies are detected with a specific cardiolipin assay.

Recent reports have shown that a relatively common mutation in the Prothrombin gene, called Prothrombin 20210, is associated with an increased risk of venous thrombosis, and this test has become routine in some settings. This mutation appears to increase the Prothrombin concentration. Finally, in the area of fibrinolysis, there are rare problems, such as hereditary Plasminogen deficiency, that cause clotting problems; however, screening is not routinely performed. Concerning other common abnormalities in levels of either profibrinolytic or antifibrinolytic factors, despite interesting research findings, none have been shown with certainty to be associated with thrombosis.

Many of the abnormalities listed above result in clinical problems in some but not in most individuals. A pattern has emerged that indicates that, by themselves, these abnormalities may carry little additional risk. However,

when they occur in the presence of other abnormalities, risk factors, or environmental exposures, the risk multiplies. Therefore, in many people with venous thrombosis, one may find several abnormalities at the same time. Environmental exposures that may add to the risk include, but are not limited to, surgery, pregnancy, cancer, and oral contraceptive or postmenopausal hormone use.

Let us consider Disseminated Intravascular Coagulation (DIC) to illustrate the concept of "consumption." DIC is a condition of generalized activation of the coagulation and fibrinolytic systems, often found secondary to a wide variety of triggering mechanisms such as certain cancers (which release procoagulant-like factors), sepsis (endotoxin and cytokine activation), tissue damage, etc. Its clinical presentation may be acute or chronic, and it can be either very active or indolent. In mild cases DIC may present few laboratory test abnormalities other than the presence of FDPs or slightly elevated D-dimer levels, but in severe cases, it may present with several factor deficiencies and bleeding or clotting problems, since many of the factors have been used up ("consumption"). This illustrates the importance and effect of balance and the difficulty in determining the underlying mechanisms in thrombotic disorders.

Platelets are clearly a key component of a proper coagulation response, and thrombocytopenia results in a risk of bleeding. A variety of drugs (the prototypes being quinine and quinidine) are associated with a relatively rare autoimmune response resulting in a drug-associated thrombocytopenia. This is presumably due to antibodies binding the drug only when it is on the surface of the platelet, and thereby accelerating platelet clearance. In most cases, the thrombocytopenia is mild, although bleeding can occur. However, heparin actually can result in a thrombotic complication, called heparin-induced thrombocytopenia (HIT). The mechanism is thought to relate to production of antibodies to heparin-Platelet Factor 4 complexes. Platelet activation and aggregation follow antibody binding, creating a hypercoagulable state. To date, heparin is the only drug that causes thrombocytopenia associated with thrombosis.

A new class of drugs currently emerging is the Glycoprotein IIb/IIIa inhibitors. These drugs block a key platelet receptor, keeping platelets from aggregating, a desirable therapeutic intervention for situations in which clotting is a major danger (e.g., during and after thrombolytic therapy in patients with myocardial infarction). Most of these drugs are associated with the rare occurence of mild thrombocytopenia. Since these drugs are designed to bind to platelets, it will be important to determine if any will exhibit a heparin-like thrombotic complication.

Laboratory Tests and Arterial Thrombosis

The first coagulation-related disorders discovered were bleeding disorders, while the next class uncovered involved venous thrombosis. Therefore, through the years, laboratory testing has focused on these problems. When in the 1970s it became clear that cardiovascular disease (CVD) is closely associated with arterial thrombosis, researchers began to study the ability to predict CVD risk with coagulation and/or fibrinolytic tests. This is an emerging area, and new findings appear frequently. At this time, no test is used routinely for this purpose. However, several points have become clear that may affect the use of these tests in the near future; they are summarized below.

None of the general coagulation screening tests (e.g., PT, aPTT, Euglobulin Clot Lysis Test, Bleeding Time) has emerged as useful in predicting those individuals at greatest risk for CVD events such as myocardial infarction or stroke. Some tests such as PT, however, are used extensively to monitor the effectiveness of oral anticoagulation. A new application in this setting is the broad use of oral anticoagulation to prevent strokes in patients with atrial fibrillation. In this setting the PT is done in a standardized manner (the INR; Table 1 from Hathaway and Goodnight).

Of the individual factors, Fibrinogen has been the most studied as a CVD risk factor. In a wide variety of populations it has become clear that individuals at the upper end of the "healthy reference range" for Fibrinogen levels have an increased risk of future CVD events (myocardial infarction, peripheral vascular disease, and stroke) compared to those at the lower end of this range. In fact, the prediction of events that occurs with Fibrinogen is independent of other CVD risk factors such as cholesterol, age, gender, diabetes status and smoking status; Fibrinogen levels yield predictive information above and beyond traditional risk factors.

It is unclear at this time why Fibrinogen levels predict future CVD events. There are two schools of thought. The first is based on the concept that Fibrinogen levels reflect the inflammatory activity (i.e., Fibrinogen is a so-called "acute phase reactant"). In the chronic setting, this would mean that Fibrinogen would be elevated in response to underlying vascular inflammation, as might occur in atherosclerosis, and reflect the atherosclerotic burden. The second school of thought is based on the concept of altered viscosity and intravascular fluid hydrodynamics. Many *in vitro* studies document the possible causative roles of high levels of Fibrinogen in CVD events, such as: increased viscosity leading to turbulence; increased platelet cross-linking leading to bigger clots

more likely to completely block an artery; or more dense clots leading to inefficient fibrinolysis. It is possible that both of these schools of thought are correct.

Studying the role of inflammation in atherosclerosis, other acute phase reactants have recently been shown to predict CVD risk. In particular, C-Reactive Protein (CRP) has been shown to be an independent risk factor. In more limited settings, low albumin levels and increased factor VIII and von Willebrand Factor (vWF) levels (all acute phase responses) have also been associated with higher risk.

Several procoagulant factors that are not acute phase reactants have been associated with CVD risk, but not in a consistent manner. For example, Factor VII (usually assessed by a coagulation activity assay) was strongly associated with future CVD risk in one large study, but not in several others. Both Factor VII and Factor VIIa levels have been shown to be elevated in patients with CVD in a variety of studies, but it is unclear whether this is cause or effect. Other markers of procoagulation, such as Prothrombin Fragment F1+2, have not proven useful in population risk prediction, most likely because the time when blood is drawn does not correspond to the time of increased thrombin generation.

While low levels of natural anticoagulants have been associated with venous thrombosis, there is little evidence that arterial thrombotic events are associated with low levels of anticoagulants. In fact, the opposite appears true, probably due to a homeostatic response to increased arterial procoagulation in people at higher risk of events. In addition, several fibrinolytic factors have been proposed as risk factors for arterial thrombosis. The most work has been done with Plasminogen Activator inhibitor-1 (PAI-1), the inhibitor responsible for moderating Tissue-Type Plasminogen activator (t-PA) activity in blood. Recent data show that endothelium-derived hemostatic factors, such as PA-I, tPA, and vWF are associated with subsequent development of first MI. For tPA, this association is independent of traditional risk factors. More data are needed to resolve this complex issue.

Another part of this emerging area is testing for increased risk due to the presence of genetic mutations in coagulation and fibrinolysis factors, just as we currently do for Factor V Leiden and Prothrombin G20210A for thrombotic venous disease. As mentioned in the Introduction, the Factor V Leiden mutation confers independent risk for arterial thrombosis in young women who are smokers. However, this is the only group in which this risk has been demonstrated. In middle-aged men and in older men and women, for example, Factor V Leiden does not appear to provide useful independent information about risk.

A large variety of relatively common mutations, or "polymorphisms" signifying their presence in a population at 1% or more, are currently being evaluated as possible risk markers that might provide improved thrombosis risk prediction over the traditional risk factors. For example, ten or more polymorphisms have been uncovered in the Fibrinogen gene. However, many of these are not independent from one another (a situation called linkage disequilibrium) and therefore do not yield independent information. Also, since they primarily affect the level of Fibrinogen, it is unclear whether they offer information beyond what is easily obtained by simply measuring the Fibrinogen level. Determining if any of these mutations provide additive risk information will take research studies involving large populations and several years.

Other factors with known polymorphisms that are being evaluated include Factor VII and PAI-1. It is anticipated that if any of these polymorphisms provides important risk information, it will be under certain conditions, similar to Factor V Leiden, where the mutation exists in the presence of the other mutations or detrimental environmental exposures. Clearly, fitting all these pieces together to provide improved risk assessment is a major challenge of the next decade. New developments will be facilitated by the information provided at an ever-increasing pace by the Human Genome Project.

Protocol for Warfarin Dosage Adjustment in Outpatients with a Target INR of 2 to 3

INR	Adjustment
1.1-1.4	*Day 1:* Add 10-20% of TWD* *Weekly:* Increase TWD by 10-20% *Return:* 1 wk
1.5-1.9	*Day 1:* Add 5-10% of TWD *Weekly:* Increase TWD by 5-10% *Return:* 2 wks
2-3	No change *Return:* 4 wks
3.1-3.9	*Day 1:* Subtract 5-10% of TWD *Weekly:* Reduce TWD by 5-10% *Return:* 2 weeks
4.0-5.0	*Day 1:* No warfarin *Weekly:* Reduce TWD by 10-20% *Return:* 1 wk
>5	Stop warfarin; monitor INR until 3.0, reinstitute at lower TWD—e.g, decrease by 20-50%. *Return:* daily

Table 1.

† Reprinted with permission from Hathaway WE, Goodnight SH. Disorders of hemostasis and thrombosis: a clinical guide. McGraw-Hill, New York, 1993, p 549.

* TWD=Total weekly dose of warfarin.

Summary

Coagulation testing continues to play a critical role in the diagnosis and risk assessment of venous thrombosis. Well-developed screening algorithms are available for most clinical diagnostic settings. Testing is also critical in therapeutic settings, including oral anticoagulation (Table 1) and heparin-induced thrombocytopenia.

Recently, with better understanding of the role of thrombosis in ischemic cardiovascular events, a new area of application has emerged: risk assessment of arterial thrombosis, particularly coronary thrombosis and stroke. While risk assessment is not yet routine, several factors involved in thrombosis show promise possibly providing important additional diagnostic and prognostic CVD information.

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ARTERIAL THROMBOSIS AND ACUTE CORONARY ARTERY SYNDROMES

The Process of Arterial Thrombosis

In normal arteries (Figure 1), the connective tissue, principally collagen, of the vessel wall is prevented from contact with platelets by the intact endothelial cell. The normal endothelium does not interact with platelets. In fact, for arterial thrombosis to occur loss of endothelial integrity must occur.

Platelets' Ia/Ib receptor allows them to adhere to collagen. In hemostasis, this receptor initiates the formation of a platelet plug to seal the damage of the vessel wall following the exposure of subendothelial components. The platelet plug recruits additional platelets to adhere; this is achieved by the binding of platelets to fibrinogen by a different receptor (IIb/IIIa). The stability of the platelet plug also depends on the generation of fibrin from fibrinogen (Figures 2,3). This involves thrombin formation, which follows release of Tissue Factor from the damaged vessel wall (Figure 4). In the evolution of the hemostatic plug, platelets adhere, then aggregate and then activate. In activation, ADP is released along with thromboxane A2 to amplify platelet recruitment to the plug (Figure 5).

These processes are beneficial in the process of hemostasis in which a plug of fibrin and platelet closes a defect in the vessel wall. These same processes, however, become pathological in the process of arterial thrombosis in which a mass of platelets and fibrin forms within the arterial lumen.

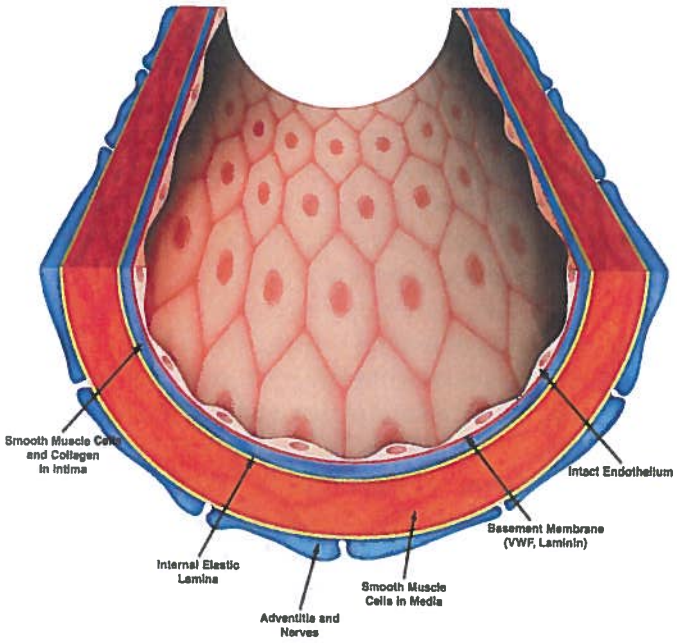

Figure 1: Normal Artery Wall.
A diagrammatic cross section of the arterial wall. The endothelial cells form a continuous sheet and adhere to a basement membrane. The endothelial cells contain von Willebrand Factor, which is released by endothelial cell damage. The intima consists of a collagen and connective tissue matrix within which are embedded smooth muscle cells. The internal elastic lamina separates the intima from the media, which contains many smooth muscle cells.

Atherosclerosis and Human Arterial Thrombosis

Normal arteries rarely undergo thrombosis: the vast majority of arterial thrombotic episodes occur in arteries that have atherosclerosis. Atherosclerosis is a complex process involving the arterial intima, characterized by the deposition of lipoproteins and cholesterol from the plasma. A striking feature of atherosclerosis is that the blood vessel is not uniformly affected with the deposits occuring in focal areas, leading to the formation of "plaques."

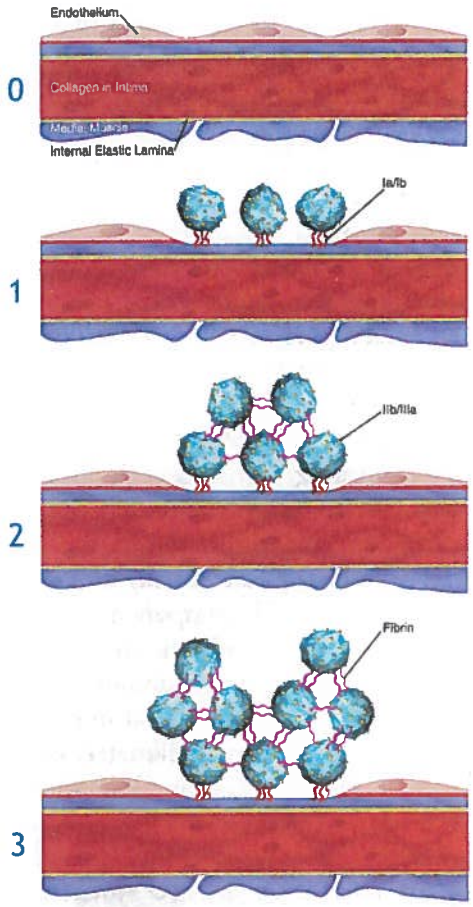

Figure 2: Endothelial Denudation Injury.
0 In the normal artery, the endothelial cells preclude contact of intimal collagen with platelets.
1 Endothelial denudation exposes collagen to which platelets adhere using the Ia/Ib receptor.
2 The platelet mass grows by platelet to platelet adhesion using the IIb/IIIa receptor and fibrinogen as a binder.
3 Fibrin formation cements the platelets into a stable mass.

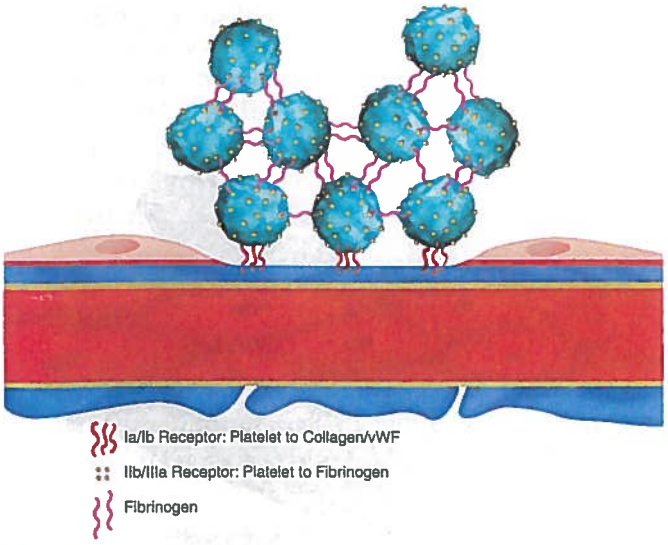

Figure 3: Platelet Adhesion and Aggregation.
Platelets adhere to exposed collagen by virtue of the Ia/Ib receptor. Adhesion of platelet to platelet to build up a small mass is by platelets expressing a receptor (IIb/IIIa) which binds to fibrinogen. The early platelet mass is unstable, with the size waxing and waning as platelets break away from the surface of the thrombus. Later, the conversion of fibrinogen to fibrin confers greater strength (cohesion) on the thrombus.

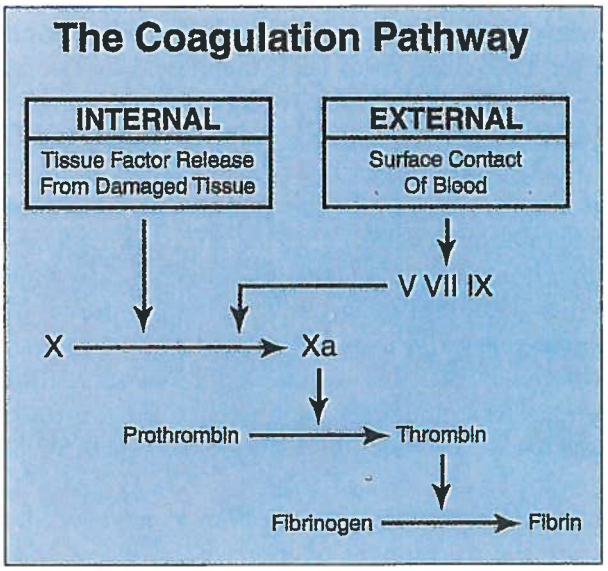

Figure 4: Coagulation.
In coagulation, the most rapid activation occurs when Tissue Factor is released from damaged tissue. This activates factor X, which then activates the generation of thrombin from prothrombin. Thrombin converts fibrinogen to fibrin. Each step amplifies the production at the next. Activation also occurs if blood meets a foreign surface, but this path is less rapid.

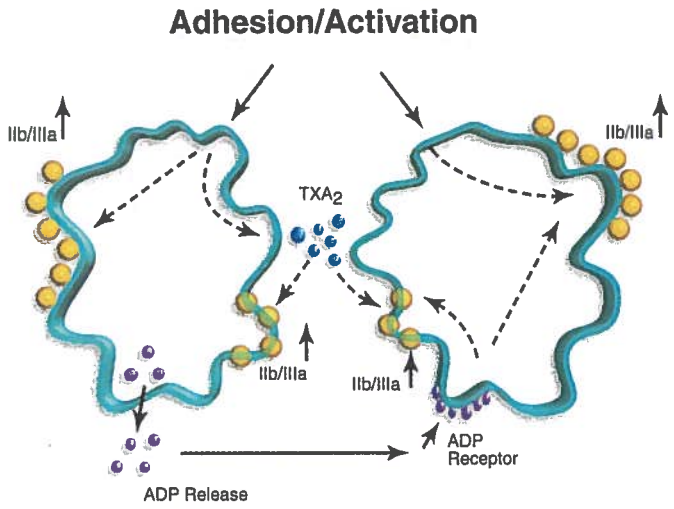

Figure 5: Platelet Activation after Adhesion/Aggregation.
Activation of platelets by thrombin or adhesion leads to a series of events, one of which is enhanced platelet surface expression of IIb/IIIa receptors. Enhanced exposure and activity of the IIb/IIIa receptors is the final common pathway of clot formation. Activated platelets release two substances that contribute to increased expression of IIb/IIIa receptors. One is thromboxane A_2 (TXA_2), which is produced via the cyclooxygenase pathway and can be blocked by aspirin. Another substance enhancing IIb/IIIa exposure is adenosine diphosphate (ADP), which interacts with ADP receptors and also stimulates platelet recruitment. ADP is inhibited by clopidogrel.

Plaque Formation

There is a sequence of events leading to plaque formation. The initial step involves modification of plasma LDL, creating an oxidative form that is a mild inflammatory mediator that enters the intima. The oxidized LDL invokes monocyte adhesion to, and migration through,

the endothelial surface. These transformed monocytes become lipid-filled foam cells, manifested as a series of yellow dots or streaks visible to the naked eye on the intimal surface. Each fatty streak is a collection of lipid-filled foam cells within the intima. The first stage of atherogenesis is now complete. To this point, endothelial denudation has not occurred, and platelet adhesion plays no part in the initiation of plaques. Although the physical integrity is not broken and there is no exposure of subendothelial collagen, the endothelial cells are not functionally normal; they may overexpress adhesion molecules; they may have impaired nitric oxide (NO) synthesis or release.

Plaque evolution from fatty streak to an advanced lesion involves the recruitment of more macrophages and the formation of a core of extracellular lipid and cholesterol within the plaque. The lipid core is thought to largely derive from the release of cytoplasmic lipid following the death of foam cells. Concomitant with core formation, smooth muscle proliferation occurs, and these cells synthesize collagen to encapsulate the lipid. Smooth muscle proliferation is driven by growth factors released by macrophages, endothelial cells, and migrating smooth muscle cells. With further evolution of the plaque, endothelial denudation occurs, and platelets are deposited, releasing PDGF (platelet-derived growth factor), another powerful stimulant of smooth muscle proliferation. The microthrombosis state is set.

Thus, once a plaque has been initiated, platelet deposition becomes a factor in plaque growth. This microthrombosis involves virtually all plaques beyond the fatty streak stage. Microthrombi have important pathophysiological implications but are far too small to obstruct flow. They are a marker of a dysfunctional endothelial surface with abnormal control of vessel tone and impaired NO synthesis. The focal endothelial injury accompanied by the microthrombi is associated with macrophage subintimal infiltration. Macrophages are activated by cytokines such as interleukin-1 (IL-1) and tissue necrosis factor alpha (TNFAlpha), which can produce free radicals as well as metalloproteinases that can degrade basement membranes and connective tissue. Macrophages also produce abundant Tissue Factor.

In experimental models, endothelial injury is also associated with high levels of serum homocysteine levels, which are an established risk factor for human atherosclerosis. The proposed mechanisms include hypercoagulability, platelet activation, and smooth muscle cell proliferation. Epidemiological evidence also indicates that certain systemic infections such as chlamydia and herpes virus family infection can enhance atherogenesis. *C. trachomatis* has been demonstrated within macrophages and endothelial cells in human plaques.

Mechanisms of Induction of Clinical Symptoms by Human Coronary Thrombus

Two distinct mechanisms are responsible for the progression of thrombi over human coronary plaques. In the first, the endothelium is damaged and thrombus forms over the plaque surface. This is called superficial or level 1 plaque injury (Figure 6). In the second level, a plaque tears open, exposing the depths of the plaque and lipid core to the circulating blood. Blood enters the lipid core itself, coming into contact with fragments of collagen, crystals of cholesterol, and Tissue Factor produced by macrophages. This is a highly thrombogenic mixture, with thrombus forming within the plaque (Figure 7). Level 3 injury follows angioplasty, in which tears extend into the media. Both endothelial erosion and plaque rupture (level 1 and 2 injury) are usually complications of plaques with a high lipid component and extensive inflammation. Endothelial erosion is an extension of the focal endothelial loss that is almost ubiquitous over established plaques. The loss of endothelial integrity leads to thrombi, ranging from a few millimeter to occluding thrombi.

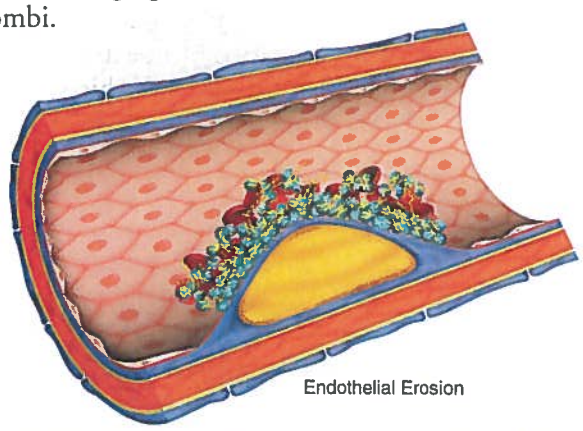

Figure 6: Arterial Thrombosis and Atherosclerosis: Endothelial Erosion. Thrombi occur over plaques either because of a superficial loss of the endothelium (erosion/denudation) or plaque rupture (disruption). In this example of erosion, thrombus is superimposed onto the surface of the plaque only.

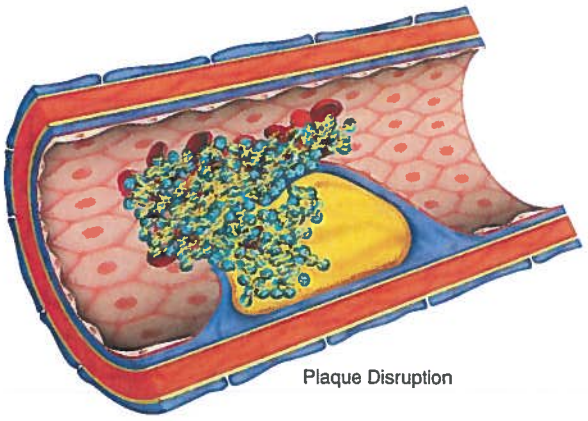

Figure 7: Arterial Thrombosis and Atherosclerosis: Plaque Rupture. In plaque rupture, the injury extends far more deeply into the intima. The fibrous cap of the plaque tears to expose the lipid core of the plaque. The lipid core contains large amounts of Tissue Factor, and thrombus forms rapidly within the plaque itself. Thrombus then grows to project into the lumen.

Vulnerability to plaque rupture is associated with a thin cap (the portion of the capsule separating the lumen from the core), with a low density of smooth muscle cells in the , and with a high density of activated macrophages in the lipid core. Rupture is thought to occur when connective tissue destruction by macrophages producing metalloproteinases exceeds the capacity of the smooth muscle cells to produce collagen to maintain the cap.

Relation of Acute Coronary Syndromes to Thrombosis

Both erosion and rupture are stimuli for thrombosis that may vary widely in magnitude. It is likely that the majority of episodes of coronary thrombosis are small and subclinical and possibly cyclic. Such episodes, however, can stimulate smooth muscle proliferation and collagen synthesis as a part of the healing process, increasing plaque size. This process is responsible for the appearance of new angiographic stenoses.

The thrombotic response that follows both erosion and plaque rupture is phasic and dynamic. With rupture, the initial thrombosis occurs within the plaque itself, which is expanded and distorted from within. At this stage, the thrombus is rich in platelets with some red cells. Toward t lumen, the thrombus becomes richer in fibrin and o n protrudes into but does not occlude the lumen. Protruding (mural), non-occluding thrombus surface is covered by a layer of activated platelets. Micro-clumps of platelets are intermittently swept downstream into the distal vascular bed (Figure 8). These clumps of platelets with high IIb/IIIa receptor expression can occlude both small arteries and arterioles in the myocardium accompanied by microscopic foci of myocyte necrosis. (Figure 9). Platelet clumps also attract an intravascular collection of polymorphonuclear leukocytes. Platelet clumps expressing enhanced levels of IIb/IIIa receptor are also found in myocardial capillaries, suggesting that activated platelets can pass back into the general circulation with the potential for systemic thrombosis. With a large enough thrombus, clinical manifestations appear. The classic pathology of unstable angina is that of a non-occluding thrombus. The phasic attacks of anginal pain that occur at rest are due to a number of mechanisms. These include bursts of platelet emboli, spasm at the site of injury in the epicardial artery, and intermittent growth of the thrombus to occlude and then rapidly reopen by natural lysis.

The outcome of thrombosis in unstable angina will de l on whether the thrombus grows to occlude the rtery or regresses with smooth muscle proliferation, eginning the healing process. The magnitude of blood low plays an important part. Plaque expansion from within causing sudden high-grade stenosis or vasospasm ccuring at the site or in the distal microvascular bed, falling flow in the epicardial artery will increase the risk of thrombosis.

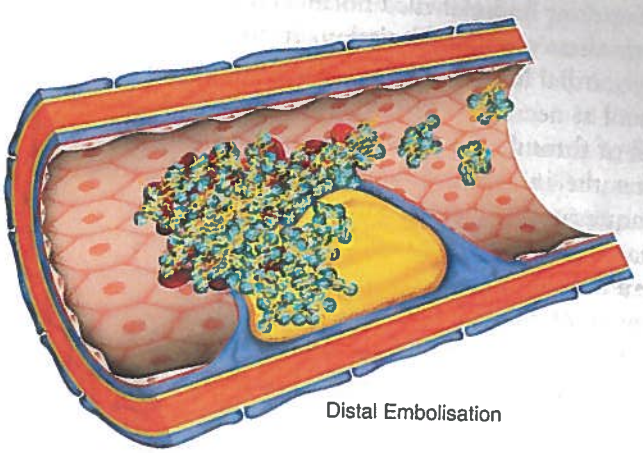

Figure 8: Arterial Thrombosis and Atherosclerosis: Distal Embolization. The surface of a thrombus, which protrudes into but does not occlude the arterial lumen, is covered by a layer of activated platelets with large amounts of the IIb/IIIa receptor exposed on their surface. The blood flow sweeps clumps of these activated platelets into the distal vascular bed.

Figure 9: Aggregated Platelets: Immunohistochemistry of IIb/IIIa Receptors. In this microscopic image, a small intramyocardial artery is blocked by a large mass of aggregated platelets which show a high level of of IIb/IIIa receptor expression (red=positive signal). Such platelet-dominated thrombi are typical of the emboli that are swept off the surface of exposed thrombi in the coronary or carotid arteries.

Coronary artery occlusive thrombosis leading to myocardial infarction may develop very rapidly or it may evolve over days. Sudden occlusive thrombosis usually reflects major disruptions of a plaque (Figure 10). However, a number of patients have a powerful response to a small plaque rupture, suggesting the importance of the systemic potential for thrombosis in determining individual outcome.

As the thrombus reaches the point of near or total occlusion, it begins to propagate in the arterial lumen, usually downstream. This thrombus has different morphological characteristics, having a high content of red cells enmeshed in a matrix of fibrin (Figure 11). Myocardial infarction implies complete occlusion long enough to lead to cell necrosis. The classic angiographic data of DeWood show that a high proportion of such arteries will

subsequently reopen by natural lysis. Fulton published work confirming the time relations of thrombosis and infarction. Radiolabelled fibrinogen was given to patients with chest pain on admission. In those who developed a myocardial infarct and died, the thrombus was studied in detail at necropsy. The study revealed that only the distal tail of thrombus was radiolabelled, showing it had grown after the infarct had occurred. The thrombus over the plaque rupture site itself was not radiolabelled, indicating that it existed before the infarction developed. The structure of the final stage of occluding thrombus with a matrix of fibrin containing trapped red cells provides a target for therapeutic fibrinolysis. A number of clinical trials confirm the benefit of thrombolysis. The earlier stage of thrombus with densely packed fibrin covered by a layer of activated platelets would, however, remain exposed for at least some days. Failure of thrombolytic therapy probably indicates major plaque disruptions with continuous thrombus formation exceeding local fibrinolysis. Angioplasty provides a means to open such arteries.

Figure 10: Occlusive Thrombus.
Major plaque disruption has resulted in complete thrombotic occlusion of a coronary artery. In this specimen, the plaque cap can still be seen, and there is a bilobed mass of thrombus. One component is in the plaque; the other component occludes the lumen.

Transmural Versus Non-Transmural Myocardial Infarction

Transmural myocardial infarction (QW-wave) consists of uniform myocardial necrosis, all of which has occurred over a short time. Epicardial artery thrombus occludes the flow suddenly and completely for at least a few hours. In contrast, non-transmural infarction is made up of many small focal areas of necrosis of widely differing ages. The example of the latter pathology is that of a non-occluding thrombus identical to that found in unstable angina in which either persistence of some antegrade or collateral flow preserves the subepicardial myocardium.

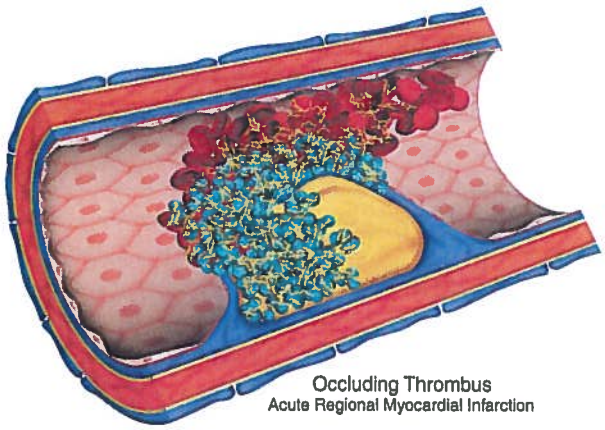

Figure 11: Evolution of Coronary Thrombi.
The substrate of the majority of major coronary thrombi is a lipid rich plaque with a core which is separated from the arterial lumen by a cap of fibrous tissue with focal areas of thinning. The earliest stage after the cap tears is thrombus within the plaque. This may remain silent and heal. The thrombus may, however, grow to protrude into the lumen and then grow further to occlude the lumen. The dynamic nature of the thrombus is important to realize; occlusive thrombus may revert to mural and vice versa over short periods of time.

Figure 12: Macropscopic View of Ulcerated Plaque.
This chronic ulcerated plaque is typical of those that occur in the carotid artery or aorta. The plaque has lost its cap, and the core lipid has washed out to leave an ulcer-like crater. Small thrombi persist on the edge of the crater and serve as a nidus for distal emboli of platelet aggregates producing, for example, transient ischemic attacks in the brain.

Aortic and Carotid Atherosclerosis and Thrombosis

Atherosclerosis is essentially identical in terms of processes in all arteries whether large or small in caliber. There are, however, some differences. Plaques in the peripheral circulation, i.e. carotid, femoral, and iliac arteries, and in the aorta are much larger. They may be up to 2 centimeters or more in their long axis. Such plaques undergo both erosion and rupture. In view of the large caliber of the peripheral arteries in comparison to the coronary vessels, occlusive thrombosis is relatively rare. Episodic erosive thrombosis over plaques in large caliber arteries leads to plaque growth and stenosis over years. The typical process involves plaque rupture, leading to protruding, masses of thrombus, which then either lyse or break off, exposing the core of the plaque. The lipid is washed out,

leaving an ulcer crater, the floor of which is covered by a layer of activated platelets (Figure 12). Such chronic subintimal ulcers may ultimately re-endothelialize but remain for long periods a source of platelet microemboli to distal sites, to the brain from the carotid arteries or emboli into the small arteries of the lower limbs from the aorta and iliac arteries.

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CLINICAL MANIFESTATIONS OF ACUTE CORONARY SYNDROMES

The term "acute coronary syndrome" (ACS) encompasses a wide spectrum of clinical presentations from unexpected sudden death to unstable angina to major Q-wave myocardial infarction. ACS accounts for nearly two million hospitalizations annually in the United States (and millions more throughout the world). The mortality of ACS is still very high and probably exceeds 25%, if one includes patients who never reach the hospital.

The underlying pathology in the coronary arteries is similar in patients with ACS. Most have significant multivessel atherosclerosis with at least one ruptured plaque with a superimposed acute thrombus. Plaque rupture and thrombosis is thought to reflect conversion of stable chronic ischemic heart disease (with or without clinical angina) to an ACS. The nature of the clinical presentation in ACS relates to the location of the plaque rupture and thrombosis, the vessel size, the severity of obstruction, the speed of occlusion, and the adequacy of collateral perfusion. In some cases, thrombosis evolves in stages with episodes of transient thrombosis and aided perhaps by vasospasm. Thus, ACS may evolve over minutes, hours or days.

Most patients who develop ACS have premonitory symptoms (the mechanism of which is uncertain). Symptoms are often nonspecific and vague, consisting of unexplained fatigue, dyspnea, and atypical chest discomfort. Many, of the patients have contacted or seen their physician during this premonitory phase, with the diagnosis of ACS missed. In addition, many patients have had undiagnosed anginal symptoms for a matter of months that have been ignored or minimized by the patient. Thus, the opportunity exists for improvement in earlier recognition of patients with ACS.

In retrospective analyses, many patients recall a high rate of unusual life stress in the weeks or months prior to clinical presentation of ACS. The stress with its high degrees of adrenergic stimulation and hormonal (cortisol) activation undoubtedly play a role in plaque rupture, enhanced thrombogenicity, and an increased likelihood of developing ventricular fibrillation in association with an acute thrombotic event. The high adrenergic tone characteristic of most ACS patients provides a rationale for acute and chronic administration of beta-adrenergic blocking agents. This class of drug reduces the occurrence of ventricular fibrillation and recurrent infarction. Studies have shown that proper use of aspirin, statins, and perhaps ACE inhibitors in patients with angina has reduced the incidence and mortality from ischemic heart disease. Coronary artery revascularization, namely bypass graft surgery in high-risk patients, has also played a major role in reducing mortality in chronic ischemic heart disease.

Sudden Death

Sudden death from ACS is caused by ventricular tachycardia or fibrillation in 80% of cases. The remainder have asystole or electromechanical dissociation. Such patients experience a fatal outcome that is not always preceded by chest pain. Although sudden death may be precipitated by strenuous physical activity, it more commonly occurs at rest, as is the case with acute myocardial infarction. A frightening statistic is that in perhaps up to 25% of cases, sudden death is the initial manifestation of ischemic heart disease. Sudden death is much more common in males.

Since the advent of sophisticated prehospital coronary care in the late 1960s, emergency medical vehicles staffed by paramedics have become available in major cities throughout the world. In the United States, pioneering systems in Seattle, Washington, and Columbus, Ohio, provided rapid response to sudden death patients, and CPR certification